UNIVERSITI TEKNOLOGI MARA

OPTIMAL STORAGE CONDITIONS FOR THE DETECTION OF Enolase DERIVED FROM THE MIDGUTS OF FIELD COLLECTED *Aedes albopictus*

By

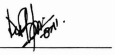
NURUL IZNI ROSMAN

Thesis Submitted in Partial Fulfillment of the Requirements for Bachelor of Medical Laboratory Technology (Hons), Faculty of Health Sciences, Universiti Teknologi MARA

2015

DECLARATION

I hereby declare that this thesis is my original work and has not been submitted previously or currently for any other degree at UiTM or any other instutitions.



NURUL IZNI ROSMAN

2011612622

JULY 2015

TABLE OF CONTENTS

ABSTRACT

OPTIMAL STORAGE CONDITIONS FOR THE DETECTION OF ENOLASE DERIVED FROM THE MIDGUTS OF FIELD COLLECTED *Aedes albopictus*

Dengue virus (DENV), is an arbovirus vectored by *Aedes albopictus*, has high tolerance in adapting to new environment including in vector and human cells. The ability of the virus to interact successfully with the protein receptor on the mosquito midgut epithelial cells contributes to the transmission of this virus to human. One of the proteins involve is enolase, which can activate plasminogen in the body leading to dengue hemorrhagic fever. In order to reduce the dengue fever cases, studies of immunology and proteomic regarding DENV need to be done for the objective to be achieved. However, improper sample collection, transportation and handling might cause the sample degraded and re-sampling during outbreak season pose risk to researcher. This study aim to determine the optimum media and the best storage conditions for midgut tissues of field collected *Ae. albopictus* by SDS-PAGE analysis. Samples of *Ae. albopictus* collected from Kolej A5, UiTM Puncak Alam were reared and adult mosquito were dissected to obtain the midgut tissues. The tissues were then preserved in phosphate buffered saline (PBS), normal saline, distilled water and 1X TAE Buffer for a week at room temperature, 4°C and -20°C. SDS-PAGE was done after a week and the result showed no expected bands at molecular weight of 57 and 67 kDa were revealed from the preserved sample. This result shows that the midgut tissues are not suitable for preservation purposes and further experiment involving protein sequencing and affinity chromatography may be used to substantiate findings.

Keywords: *Aedes albopictus*, DENV, enolase, SDS-PAGE, preservation

CHAPTER 1
INTRODUCTION

1.1 Background of the study

Aedes albopictus, (alternative name, Asian tiger mosquito) is a well-recognized vector for arboviruses such as dengue viruses (DENV), chikungunya viruses (CHIKV) and possibly West Nile virus (WNV) (Bonizzoni *et al.*, 2013). Due to its high tolerance in adapting new habitat, DENV, which vectored by *Aedes albopictus*, has become worldwide concern as increasing of dengue fever disease were reported each year. Each of DENV serotypes causes different severe reaction in their infected host (mainly human). DENV serotype 1 (DENV-1) and DENV-3 causes severe reaction in primary infection while secondary infection is more severe if host was infected by DENV-2 and DENV-4 (Patramool *et al.,* 2011). As a secondary vector of DENV, the virus tropism is mainly in midgut (MG) and salivary glands of the mosquito. Midgut is the first barrier that pathogens must overcome to establish infection in insects (Saboia-Vahia *et al.*, 2012) before heading to the salivary glands for disease transmission to human. In midgut, there are some protein receptors which can be identified by DENV, which will be useful for future proteomic studies.

Proteins in mosquito are essential as it is the major nutrient necessary for oogenesis in female mosquito (Pitts, 2014). Other than that, protein in mosquito are vital for mosquito's development by which providing energy, act as defense mechanism against pathogens and involve in mosquito's metamorphosis (Saboia-Vahia *et al.*, 2014). DENV virus dependent on protein receptors vector/host cell receptor to initiate infection. The binding of the virus to these receptors are affected by abundance and distribution of the receptor throughout the vector/host cell surface (Cruz-Oliveira *et al.,* 2014). Studies have found that interactions between virus and